

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 November 2002 (21.11.2002)

PCT

(10) International Publication Number
WO 02/093152 A1

- (51) International Patent Classification⁷: G01N 27/327, 33/50, C12Q 1/26
- (21) International Application Number: PCT/US02/15706
- (22) International Filing Date: 15 May 2002 (15.05.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/291,361 16 May 2001 (16.05.2001) US
60/377,886 2 May 2002 (02.05.2002) US
10/146,577 14 May 2002 (14.05.2002) US
- (71) Applicant: THERASENSE, INC. [US/US]; 1360 South Loop Road, Alameda, CA 94502 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (72) Inventor: HELLER, Adam; 4711 Spicewood Springs Road, #271, Austin, TX 78705 (US).
- (74) Agent: DE RUNTZ, K., Alison; Skjerven Morrill LLP, Three Embarcadero Center, 28th Floor, San Francisco, CA 94111 (US).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/093152 A1

(54) Title: METHOD FOR THE DETERMINATION OF GLYCATED HEMOGLOBIN

(57) Abstract: A method of determining the percentage of glycated hemoglobin in a blood sample is disclosed wherein at least one of the assay steps is performed electrochemically. The method includes determining the total amount of hemoglobin in a sample by electrochemically measuring, in an oxygen electroreduction reaction at a cathode, the amount of oxygen in the sample. Because the amount of oxygen dissolved in the sample is known, the total hemoglobin is determined by subtracting the amount of free oxygen from the total oxygen measured, recognizing the fast equilibrium $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$. This can be followed by determining the amount of glycated hemoglobin in the sample. The cathode reaction is accomplished by contacting the sample with an enzyme, the enzyme being a copper-containing enzyme having four copper ions per active unit. The family of these enzymes includes, for example, laccases and bilirubin oxidases.

METHOD FOR THE DETERMINATION OF GLYCATED HEMOGLOBIN

BACKGROUND

[0001] Field of the Invention

[0002] The present invention relates to a process for the determination of the amount of irreversibly glycated hemoglobin, or HbA1c, present in a sample of blood, relative to the amount of total hemoglobin. In particular, the invention incorporates in the method an electrochemical, enzyme-catalyzed reaction or reactions.

[0003] Background Information

[0004] HbA1c is a glycated hemoglobin formed by a binding reaction between an amine group of hemoglobin and the glucose aldehyde group, for example between the amino group of the N-terminal valine of the β -chain of hemoglobin and the glucose aldehyde group. The binding reaction first forms a Schiff's base and then a stable ketoamine by Amadori rearrangement. The percentage of HbA1c (i.e. the amount of glycated hemoglobin relative to total hemoglobin in the blood) has come to be taken as a measure of the level of blood glucose control a diabetic patient has maintained for a period of two or three months prior to the measurement. As such, percentage HbA1c has become an important measurement by which health care providers can assist diabetic patients in their care.

[0005] There are many known assays that can be used to determine HbA1c percentage. In recent years research efforts have focused on creating assays that are both highly accurate and fast. However, known HbA1c assays typically require a substantial number of time-consuming steps wherein the blood components must be separated and treated.

[0006] In the health care context, a diabetic patient is typically guided by a physician to obtain an HbA1c measurement when the physician realizes that there is a need for such information during an office visit. The patient then provides a blood sample to a laboratory and results are returned to the physician hours or days later. Typically, the lab will use a table top analyzer of the type presently available commercially. This time lag between the patient's visit and the result of the test requires that the physician review the result long after the patient has left the office. If the physician believes that further consultation with the patient is required in light of the test result, the patient must be contacted again.

[0007] Currently, there is a device sold under the name "A1c NOW" by Metrika, Inc. of Sunnyvale, California. This handheld and disposable device (based on technology described in U.S. Patent No. 5,837,546 entitled "Electronic Assay Device and Method," incorporated herein by reference) is said to provide an HbA1c test result in eight minutes using a relatively small sample of blood. The A1c NOW device is an example of the market demand for a fast method of providing an HbA1c result for either home or doctor's office use. However, the A1c NOW device is not as accurate as some laboratory assays. Thus, research has continued to focus on finding a highly accurate HbA1c assay that is also fast enough and simple enough to permit a diabetic and his or her doctor to take a blood sample during an office visit and have a trustworthy HbA1c measurement available for discussion in the same visit.

SUMMARY OF THE INVENTION

[0008] The present invention comprises a method of determining the amount or percentage of glycated hemoglobin in blood or a sample derived from blood, in which at least one of the assay steps is performed electrochemically. The use of electrochemical methodology can retain or improve the accuracy of other methods and potentially speed the ultimate determination. Devices providing electrochemical measurements can also be relatively small.

[0009] In one embodiment, the method includes electrochemically determining the total amount of hemoglobin in a sample by electrochemically measuring, in an

oxygen electroreduction reaction at a cathode, the amount of oxygen in the sample, preferably after it was exposed to air so as to assure that the hemoglobin is oxygenated. Because the amount of oxygen dissolved in aerated physiological buffer at the assay temperature in the absence of hemoglobin, termed here free oxygen, is known, the total hemoglobin may be determined by subtracting the amount of free oxygen from the total oxygen measured, recognizing the fast equilibrium $\text{Hb} + \text{O}_2 \leftrightarrow \text{HbO}_2$. Electrochemically determining the total hemoglobin value can be followed by a determination of the amount of glycated hemoglobin in the sample. In the process of the invention, the cathode reaction is accomplished by contacting the sample with an enzyme. In this embodiment, the enzyme can be a copper-containing enzyme, containing four copper ions per active unit. The family of these enzymes includes, for example, laccases and bilirubin oxidases.

[0010] The glycated hemoglobin can be determined in different ways. In one embodiment, the glycated hemoglobin is separated from the sample, for example by capturing it with immobilized antibodies against HbA1c or with a boronic acid modified surface. Examples of surfaces include those of small magnetic, polymer or glass beads. The percentage HbA1c can then be determined by either measuring the hemoglobin left in the sample from which the HbA1c has been removed, or by measuring the amount of glycated hemoglobin in the separated portion of the sample. The amount of glycated hemoglobin can be measured spectrophotometrically, or by an electrochemical measurement in the same manner as the total hemoglobin. In another embodiment the hemoglobin is hydrolyzed by an established method, such as digestion with a proteolytic enzyme. The ketoamines in the hydrolyzate, such as the fragments comprising the Amadori rearrangement products of the Schiff base formed of amino acids, including valine and glucose, are then determined, preferably by an electrochemical method. In the electrochemical method, the electrooxidation of the hydrolyzed Amadori rearrangement product may be catalyzed by an enzyme and a dissolved or immobilized redox mediator. The enzyme can be, for example, a fructosamine oxidase, a four copper-ion containing copper enzyme such as a laccase or a bilirubin oxidase, ceruloplasmin, or ascorbate oxidase. The redox mediators can be, for example, complexes of $\text{Os}^{2+/3+}$, or of $\text{Ru}^{2+/3+}$.

DETAILED DESCRIPTION

[0011] The invention incorporates one or more electrochemical steps in the method of determining percentage HbA1c. The method of the invention is based on the understanding that hemoglobin, being the oxygen carrier of blood, reversibly binds oxygen, forming HbO₂. The equilibrium $\text{Hb} + \text{O}_2 \leftrightarrow \text{HbO}_2$ is rapid. Because O₂ is rapidly released by HbO₂ when O₂ is depleted from the solution in an electrochemical cell, it is possible to determine the concentration of HbO₂ in light of the reaction $4\text{H}^+ + 4\text{e}^- + \text{HbO}_2 \rightarrow 2\text{H}_2\text{O} + \text{Hb}$.

[0012] Determining Total Hemoglobin Electrochemically

[0013] In the invention, it may be useful to pre-treat a blood sample by collecting the relatively large blood cells on a filtration membrane. After rinsing the collected cells with saline to remove adhering proteins, the cell membranes may be ruptured by exposing them to de-ionized water or a detergent. In this manner, the dissolved hemoglobin will pass the filtration membrane. The cell membranes will remain on the filter paper.

[0014] In a preferred form of the invention, total hemoglobin is then determined from the sample by electroreducing the oxygen bound to the hemoglobin to water at the cathode in an electrochemical cell. The oxygen electroreduction catalyst preferably comprises a so-called "copper" enzyme such as bilirubin oxidase, a laccase, or an ascorbate oxidase.

[0015] The catalyst may further comprise a redox mediator to form a "wired enzyme" arrangement. In this system, the electrical connection is between a cathode of the electrochemical cell and the oxygen reduction catalyzing enzyme, especially a copper-containing enzyme, such as bilirubin oxidase (sometimes referenced herein as BOD). Thus, in one form of the invention, it is preferred to "wire" reaction centers of an enzyme, e.g. bilirubin oxidase, to a cathode. Bilirubin oxidase catalyzes the four-electron reduction of oxygen to water. A cathode constructed with bilirubin oxidase is especially preferred as the redox enzyme can function under relatively neutral pH conditions. However, other enzymes (e.g. laccase) may be useful in the method of the invention so long as they provide catalytic functionality for the reduction of oxygen to

water.

[0016] Thus, the concentration of HbO_2 can be measured by the reaction $4\text{H}^+ + 4\text{e}^- + \text{HbO}_2 \rightarrow 2\text{H}_2\text{O} + \text{Hb}$. This measurement may be done coulometrically. The concentration of available oxygen in arterial blood tends to be about 8 mM. Because the concentration of O_2 in water in equilibrium with air at 25°C is known (the concentration is generally around 0.24 mM), the amount of non-Hb bound O_2 can then be subtracted in calculating the amount of HbO_2 .

[0017] A cathode useful in the invention effectuates the four-electron electroreduction of O_2 to water. The blue, copper-containing oxidases, examples of which include laccases, ascorbate oxidase, ceruloplasmine, and bilirubin oxidase, catalyze the four-electron reduction of O_2 to water. The preferred enzymes are exemplified by bilirubin oxidases, which unlike laccases, retain their more than 80%, and usually retain more than 90%, of the maximal activity under physiological pH. The catalytic reduction of O_2 to water depends on the coordination of the four Cu^{+2+} ions of the enzymes. The Cu^{+2+} ions are classified, by their ligands, into three "types", types 1, 2, and 3. Type 1 Cu^{+2+} centers show an intense Cys S to Cu(2) charge transfer band at around 600 nm; the site accepts electrons from an organic substrate, such as a phenol, ascorbate, or bilirubin, and relays the electrons to the O_2 -reduction site. The O_2 -reduction site is a tri-nuclear cluster, consisting of one type 2 Cu^{+2+} center and a pair of type 3 Cu^{+2+} centers, their spectrum showing a shoulder at 330 nm.

[0018] There are different forms of bilirubin oxidase available, such as bilirubin oxidase from *Myrothecium verrucaria* (*Mv*-BOD) and bilirubin oxidase from *Trachyderma tsunodae* (*Tt*-BOD). Bilirubin oxidases are usually monomeric proteins and have molecular weights approximately ranging from about 52 kDa to about 65 kDa. *Tt*-BOD is a monomeric protein with a molecular weight of approximately 64 kDa, while that of *Mv*-BOD is about 52 kDa. Both *Mv*-BOD and *Tt*-BOD are multicopper oxidases, each containing one type 1, one type 2, and two type 3 copper ions. These three types are defined by their optical and magnetic properties. Type 1 (blue) copper ions have a characteristic Cys to Cu (2) charge-transfer band near 600 nm. The type 1 copper center accepts electrons from the electron-donating substrate of the enzyme and relays these to the O_2 reduction site. The latter is a trinuclear

cluster, consisting of a type 2 copper ion and a type 3 pair of cupric ions with a characteristic 330 nm shoulder in its absorption spectrum.

[0019] In one embodiment of the invention, bilirubin oxidase from *Myrothecium verrucaria* could be used in a cathode electrocatalyst layer. In a cathode constructed using *Mv*-BOD, the electrostatic adduct of the poly-anionic *Mv*-BOD and its "wire", the polycationic redox copolymer of polyacrylamide (PAA) and poly (N-vinylimidazole) (PVI) complexed with $[\text{Os} (4,4'\text{-dichloro-2,2'}\text{-bipyridine})_2\text{Cl}]^{+2+}$, are immobilized on the cathode.

[0020] In another embodiment of the invention, bilirubin oxidase (BOD) from *Trachyderma tsunodae* can be used in a cathode electrocatalyst layer. In *Tt*-BOD all of the ligands of the Type 2 and Type 3 Cu^{+2+} centers are His (histidines), similar to ascorbate oxidase. It is believed that the full histidine coordination of the type 2 Cu^{+2+} center is the underlying cause of the relative insensitivity of bilirubin oxidases to inhibition by the chloride and hydroxide anions (as are found at physiological concentration). Accordingly, it is expected that other enzymes having the three types of copper centers would also be useful as components of cathode electrocatalysts in cathodes operating under at near neutral pH.

[0021] The redox potentials of the redox polymers that "wire" the cathode enzyme can be tailored for use in the invention. Redox polymers for use in the method may include PAA-PVI- $[\text{Os}(4,4'\text{-dichloro-2,2'}\text{-bipyridine})_2\text{Cl}]^{+2+}$ which can be prepared as follows: 4,4'-Dinitro-2,2'-bipyridine *N,N'*-dioxide was prepared as described in Anderson, S.; Constable, E. C.; Seddon, K. R.; Turp, E. T.; Baggott, J. E.; Pilling, J. J. *Chem. Soc., Dalton Trans.* 1985; 2247-2250, and Kenausis, G.; Taylor, C.; Rajagopalan, R.; Heller, A. *J. Chem. Soc., Faraday Trans.* 1996, 92, 4131-4135. 4,4'-dichloro-2,2'-bipyridine (dcl-bpy) was synthesized from 4,4'-dinitro-2,2'-bipyridine *N,N'*-dioxide by modifying the procedure of Maerker et al. (see Anderson, S., *supra* and Maerker, G.; Case, F. H. *J. Am. Chem. Soc.* 1958, 80, 2475-2477.). $\text{Os}(\text{dcl-bpy})_2\text{Cl}_2$ was prepared as follows: $(\text{NH}_4)_2\text{OsCl}_6$ and "dcl-bpy" were dissolved in ethylene glycol in a 1:2 molar ratio and refluxed under argon for 1 hour (yield 85%). The $\text{Os}(\text{dcl-bpy})_2\text{Cl}_2$ was then complexed with the 1:7 polyacrylamide-poly(*N*-vinylimidazole) (PAA-PVI) copolymer and purified as described in Zakeeruddin, S.

M.; D. M. Fraser, D. M.; Nazeeruddin, M.-K.; Gratzel, M. *J. Electroanal. Chem.* 1992, 337, 253-256 to form the PAA-PVI-[Os(4,4'-dichloro-2,2'-bipyridine)₂Cl]⁺²⁺ redox polymer. Those skilled in the art are aware of numerous variations that can be prepared and used as redox polymers according to the invention.

[0022] Determination of the HbA1c Percentage

[0023] Once the total hemoglobin has been measured, the HbA1c/Hb ratio can be determined by separating the HbA1c fraction from the sample. The HbA1c, which can be converted to HbA1cO₂, can then be measured indirectly and electrochemically using the same method as for the total hemoglobin. Alternatively, these fructosyl amines may be subject to direct enzyme catalyzed electro-oxidation, for example using fructosyl amine oxidases having FAD/FADH reaction centers, or by one of the copper enzymes.

[0024] The following are examples of suitable methods which incorporate the separation and HbA1c assay steps.

[0025] Example 1. Affinity gel columns can be used to separate bound, glycosylated hemoglobin from the nonglycosylated fraction. The gel contains immobilized *m*-aminophenylboronic acid on cross-linked, beaded agarose. The boronic acid first reacts with the *cis*-diol groups of glucose bound to hemoglobin to form a reversible 5-membered ring complex, thus selectively holding the glycosylated hemoglobin on the column. Next, the nonglycosylated hemoglobin is eluted. The ring complex is then dissociated by sorbitol, which permits elution of the glycosylated hemoglobin. Using affinity chromatography, absorbances of the bound and nonbound fractions, measured at 415 nm, are used to calculate the percent of glycosylated hemoglobin.

[0026] Example 2. Magnetic beads that are < 1 μm (available from Bangs Laboratories), on which antibodies against HbA1c would be immobilized, can be mixed with a citrate-solution diluted blood sample. Two measurements are performed, one on the entire sample, and a second on the re-oxygenated Hb1Ac bound to the magnetic beads, after their removal to a chamber of an electrochemical cell. Alternatively, the second measurement can be on the residual Hb, after the

magnetic separation of the bead-bound HbA1c.

[0027] Example 3. Two samples of the lysed red blood cells in citrate buffer can be coulometrically assayed in two chambers. In Chamber 1, the total HbO₂ would be measured. Chamber 2 contains the immobilized HbA1c-specific antibody. Either of the two would capture HbA1c without capturing Hb. After rinsing or passage of citrate buffer through Chamber 2 (e.g. by repeated filling through capillary action and touching the edge of the chamber to filter paper), the chamber would contain only HbA1cO₂. The HbA1cO₂ would be assayed electrochemically (preferably coulometrically) by its electroreduction, $4H^+ + 4e^- + HbA1cO_2 \rightarrow 2H_2O + HbA1c$. The HbA1c/Hb ratio can then be calculated from the two coulometric measurements.

[0028] Example 4. As in example 3 above, except that the two coulometric measurements would be performed in a single chamber. The chamber, which would contain the immobilized HbA1c capture agent, would be filled with a citrate solution of the lysed red blood cells. First, the total HbO₂ would be electrochemically (preferably coulometrically) measured. Next, the unbound Hb, but not the bound HbA1c, would be rinsed out, the HbA1c would be re-equilibrated with air, and its amount would be coulometrically measured.

[0029] Thus, the assay of the invention, in one form, can comprise a method of determining the ratio of HbA1c to total Hb in blood, the method comprising obtaining a blood sample; electrochemically determining the total amount of hemoglobin in the sample, or in a treated portion of the sample; electrochemically determining the amount of HbA1c in the sample; and calculating the ratio of HbA1c to total hemoglobin. In a preferred form the method of electrochemically determining the total amount of hemoglobin in the sample is accomplished by placing the sample in an electrochemical cell in which, at the cathode, a cathode enzyme is bound, for example using a redox polymer. In this method, it is preferred that the enzyme be a laccase or a bilirubin oxidase which will electroreduce oxygen bound to the hemoglobin to water. The hemoglobin content is determined from the oxygen content.

[0030] In another form of the invention the electrochemical determination of HbA1c fraction can be accomplished by one of two methods. In a first method, the A1c containing fraction of the hemoglobin is separated by physical means, such as by

use of an HbA1c specific antibody. Under appropriate conditions the HbA1c then present in the form of HbA1cO₂ can then be electrochemically determined by electroreduction of the oxygen (again with an enzyme selected to accomplish the four electron reduction of oxygen). In a second method, the glycated protein (a fructosyl amine) can be directly oxidized on cross-linked poly(N-vinyl imidazole) based redox polymer films (without an enzyme) of sufficiently positive oxidizing potential. Alternatively, enzymatic electrooxidation of the fructosyl amines can be used for this part of the determination.

[0031] Finally, the invention comprises an electrochemical method for the determination of HbA1c (or HbA1c/Hb ratio) comprising determining from a starting sample, in an electrochemical cell, the total amount of hemoglobin (e.g. by measuring bound oxygen), separating the HbA1c component from the sample using an HbA1c capturing agent, and measuring hemoglobin content in the captured or non-captured portion of the sample.

[0032] Devices for accomplishing the method of the invention are preferably small. By incorporating electrochemical steps, it may be possible to prepare biosensor strips which include a cathode at which the chemistry discussed herein is placed, as well at which the necessary anode is constructed. Such strips can be prepared using techniques presently used for making commercially available biosensor strips that are used for glucose determinations, such as the FreeStyle blood glucose system sold by TheraSense, Inc. Samples could then be applied to these strips and the strips placed in the measuring instrument (meter) to be "read." By constructing a portion of the equipment in the form of electrochemical biosensor strips, the electrochemical method of the invention provides a significant potential advantage of creating a smaller analysis device while providing accurate results.

CLAIMS

What is claimed is:

1. A method for determining the percentage of glycated hemoglobin in a fluid sample comprising:
 - electrochemically determining the total amount of hemoglobin in a blood sample;
 - determining the amount of glycated hemoglobin in the sample; and
 - calculating the ratio or percentage of glycated hemoglobin to total hemoglobin.
2. The method of claim 1, wherein electrochemically determining the total amount of hemoglobin comprises:
 - contacting the sample in an electrochemical cell with an enzyme capable of determining the oxygen content of the sample; and
 - calculating the amount of hemoglobin from the oxygen content.
3. The method of claim 2, wherein the enzyme is a copper-containing enzyme.
4. The method of claim 2, wherein the enzyme is selected from the group consisting of laccase, bilirubin oxidase, and ascorbate oxidase.
5. The method of claim 1, wherein determining the amount of glycated hemoglobin is performed spectrophotometrically.
6. The method of claim 1, wherein determining the amount of glycated hemoglobin is performed electrochemically.

7. The method of claim 1, wherein determining the amount of glycated hemoglobin is performed by direct enzymatic action.

8. The method of claim 1, wherein determining the amount of glycated hemoglobin is performed by direct enzymatic reaction.

9. A method for determining the percentage of glycated hemoglobin to total hemoglobin in blood comprising:

electrochemically determining the total amount of hemoglobin in a blood sample;

separating the glycated hemoglobin from the sample to form a second sample;

determining the amount of glycated hemoglobin in the second sample; and

calculating the percentage of glycated hemoglobin to total hemoglobin in the sample.

10. The method of claim 9, wherein determining the amount of glycated hemoglobin in the second sample is done electrochemically.

11. The method of claim 9, wherein determining the amount of glycated hemoglobin in the second sample is done spectrophotometrically.

12. A method for determining the percentage of glycated hemoglobin to total hemoglobin in blood comprising:

electrochemically determining the total amount of hemoglobin in a blood sample by exposing the sample to an enzyme associated with a cathode, determining the amount of oxygen from an enzymatic redox reaction, and calculating the amount of hemoglobin from the amount of oxygen;

determining the amount of glycated hemoglobin in the sample; and

calculating the percentage of glycated hemoglobin to total hemoglobin in the sample.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/15706

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 27/327, 33/50; C12Q 1/28

US CL : 436/66, 67, 138, 151; 435/14, 25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/66, 67, 138, 151; 435/14, 25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,126,247 A (PALMER et al) 30 June 1992, entire document.	1-12
A	US 5,609,749 A (YAMAUCHI et al) 11 March 1997, entire document.	1-12
A	US 5,807,747 A (WALLWORTH et al) 15 September 1998, entire document.	1-12
A	US 4,820,636 A (HILL et al) 11 April 1989, entire document.	1-12
A	EP 0,745,843 A2 (PARK et al) 12 April 1996, entire document.	1-12

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

28 JUNE 2002

Date of mailing of the international search report

19 JUL 2002

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JEFFREY R. SNAY

Telephone No. (703) 308-0661